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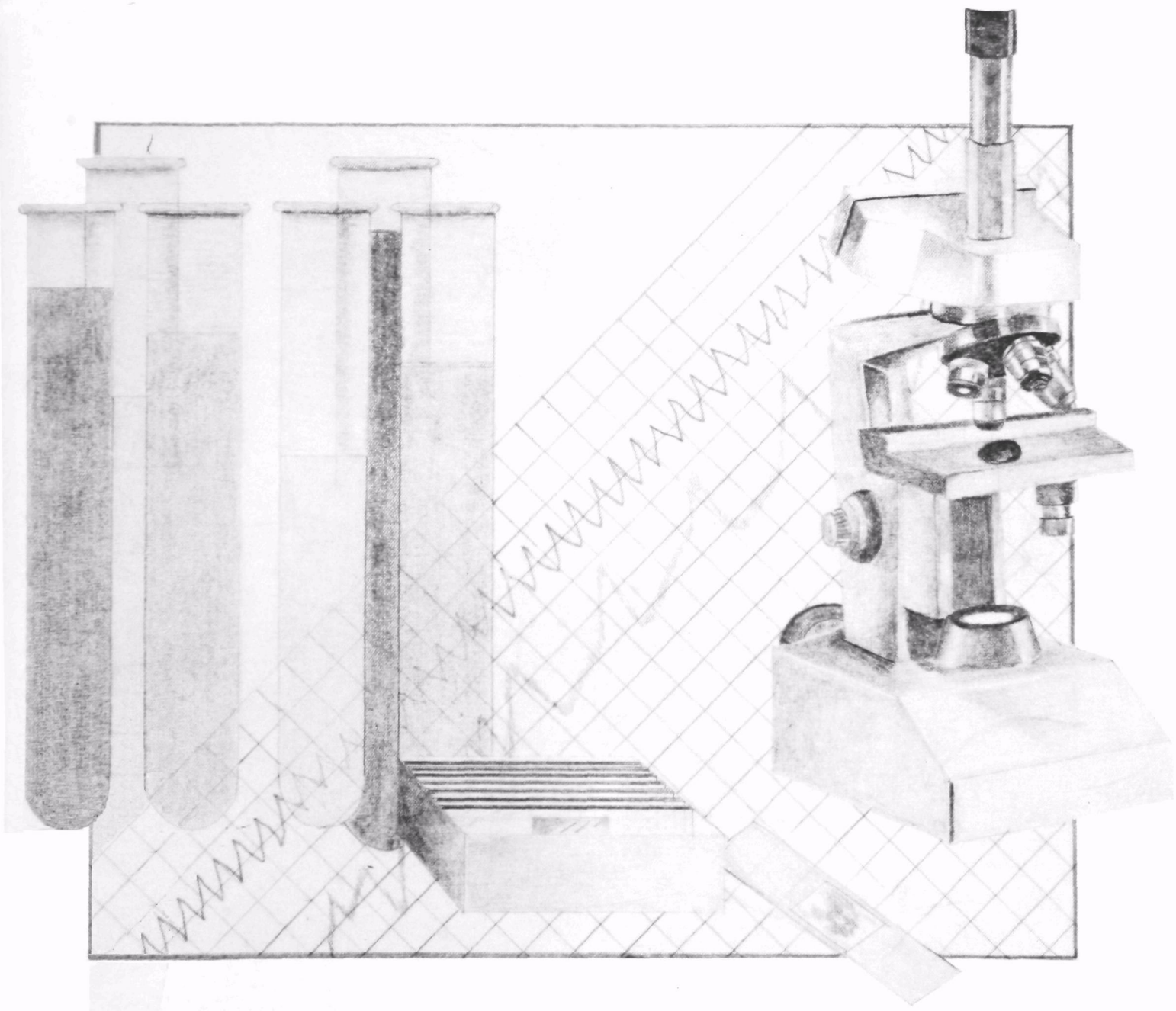
Hazard Evaluation Division Standard Evaluation Procedure

Toxicity Potential (Guidance for Analysis and Evaluation of Subchronic and Chronic Exposure Studies)



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HAZARD EVALUATION DIVISION

STANDARD EVALUATION PROCEDURE

TOXICITY POTENTIAL:

GUIDANCE FOR ANALYSIS AND EVALUATION OF SUBCHRONIC AND CHRONIC EXPOSURE STUDIES

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STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.

John W. Melone, Director
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Preamble

Of all the chemicals to which humans might be exposed, pesticides are unique by reason of their deliberate introduction into the environment to kill or otherwise control life forms considered detrimental to human welfare. Experimental animals have served as useful models for detection of potential human responses to these poisonous substances. The Environmental Protection Agency has published regulations relating to acceptable practices for conducting and reporting animal studies[1], as well as guidelines[2] that suggest acceptable and useful experimental designs (protocols) for evaluation of adverse health effects (hazards) relating to pesticidal agents.

The subchronic oral study has been designed to permit determination of toxic effects associated with repeated exposure for a period of 90 days[3]. This type of study can provide information relating to toxic effects and potential health hazards likely to arise from repeated exposures over a limited time period. Data from this type of study are also useful in predicting potentially important toxicity end points, identifying potential target organs and systems, and in establishing the dose regimen in chronic exposure studies.

The objective of chronic exposure studies [4] is the determination of toxic effects and potential health hazards following prolonged, repeated exposure. This type of study is generally used for substances, and sometimes their metabolic or

breakdown products, when repeated exposure to humans is likely to take place over a significant portion of their life span as is potentially the case with pesticide residues in the diet.

The purpose of this document is to present a very general guidance framework for analysis and evaluation of data from subchronic and chronic dietary exposures of rodents to pesticidal agents. It does not pretend to take the place of or mimic the many excellent texts on the subjects of toxicology, clinical chemistry and pathology, nor does it attempt to consider all specific effects and the multiplicity of effect patterns likely to be encountered in subchronic or chronic exposure studies. However, what is discussed is equally applicable to studies using other continuous routes of exposure, other species, and other types of chemical agents.

This document can and should be used in concert with the Core Classification system in determining study acceptability. The proper use of the Core Classification system requires an understanding of the underlying basis for the various Core "requirements" and assumes a knowledge of which study parameters should be construed as requirements and which are merely suggestions. Guidance is provided in this document on such topics as the Maximum Tolerated Dose, the No Observed Effect Level, and the utility of analyzing blood and urine.

A definition of chemical oncogenicity and discussion of implications pertaining thereto are presented by Paynter[8]. This definition and discussion should be considered as part of the guidance offered by this document.

I. Analysis and Evaluation of Adverse Effects in Experimental Animal Subchronic and Chronic Exposure Studies

A. Definitions and Concepts

Both subchronic and chronic exposure studies, regardless of routes of administration, share many common toxicity end points used for hazard identification and risk assessment.

Prior to discussion of these end points, some comment on terms and concepts presented in this document is appropriate. Toxicity means the intrinsic capacity of a chemical substance or a mixture of substances to induce injury. Hazard means the observed toxic manifestation(s) induced by a known quantity or quantities of a substance under known exposure conditions. Risk means the probability that the identified hazard(s) will or will not be encountered under anticipated exposure conditions. The identification of hazard and assessment of the risk potential of a given substance are informed judgments. Such judgments are usually based on data relating to toxicity, proposed uses, and anticipated exposure conditions. Use and expected exposure conditions define the type, probable duration and quantity of exposure, as well as the size and composition of the exposed population. A particular pesticide product may have one or several potential risks depending on use(s) and attendant exposure conditions.

The relationship of toxicity, hazard, and risk was perhaps first articulated by Paracelsus (1493-1541) as, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy."[5] In 1975 the National Academy of Sciences restated this principle thus, "A chemical--any chemical--is a poison only as a consequence of the quantity with which the host must deal."[6] This concept is a fundamental principle of toxicology and hazard assessment. The risk of a pesticide to man and the environment is related to exposure conditions and cannot be rationally equated per se with the intrinsic toxicity of any substance. To illustrate this point imagine two containment systems: (a) a perfect system which absolutely prevents any exposure of man and the environment to a substance having a dermal or oral toxic dose of 0.001 ug/kg of body weight and (b) an extremely imperfect system which allows high human and environmental exposure to the same substance. In system (a) the exposure is zero and the risk to man and the environment is also zero although the toxicity of the substance remains unchanged. In system (b) the exposure is potentially large and the risks of intoxication and other adverse effects to man or the environment are potentially very great.

The term dose refers to a stated quantity or concentration of a substance to which an organism is exposed and dose-response-relationship means the correlative association existing between the dose administered and the response (effect) or spectrum of

responses that is obtained. The concept expressed by these latter terms is indispensable to the identification, evaluation, and interpretation of most pharmacological and toxicological responses to chemicals. It is therefore important to understand the basic assumptions which underlie and support the concept.

The primary assumption is that a dose-response-relationship is firmly based on knowledge or a defensible presumption that the response (effect) observed is a result of exposure to a known substance. Correlative assumptions are: (a) the observed response is a function of the concentration at a site, (b) the concentration at a site is a function of the dose, and (c) response and dose are causally related.[7]

The essential purpose of chronic exposure studies is the detection of valid biological evidence for a toxic and/or an oncogenic potential of the substance being investigated. Therefore, protocols should maximize the sensitivity of the test without significantly altering the accuracy and interpretability of the biological data obtained. The dose regimen has an extremely important bearing on these two critical elements. The concept of the maximum tolerated dose (MTD) has had a significant influence on the selection of doses for long-term (chronic) exposure studies and on the interpretation of observed dose responses. This subject has been discussed in relationship to oncogenicity data bases.[8]

Conscientious attempts to accommodate the MTD concept in chronic studies, regardless of species used, have frequently caused dose level adjustments in one or more animal groups and these have frequently introduced interpretational difficulties at the termination of the study. Misinterpretation of the intent of the MTD concept has occasionally caused the invalidation of an otherwise valid study or has caused its classification to be inappropriately reduced when applying the Core Classification scheme criteria. Therefore, the characteristics of the highest dose to be used in modern chronic exposure studies should be reconsidered and more clearly defined.[8,9] Ideally, the dose selection for chronic studies should maximum the detection of potential dose response relationships and facilitate the extrapolation of these to potential hazards for other species including humans. Therefore the largest administered dose, the MTD, should be one which produces signs of <u>minimal</u> toxicity that do <u>not</u> compromise biological interpretability of the observed responses. For example, the upper dose should not: a) alter survival in a significant manner due to effects other than tumor production; b) cause a body weight decrement from concurrent control values of greater than 10-12%; c) exceed 5% of the total diet because of potential nutritional imbalances caused at higher levels or; d) produce severe toxic, pharmacologic or physiologic effects that might shorten duration of the study or otherwise compromise the study results.

Although it can be argued that responses observed at doses far in excess of levels experienced under real or potential exposure conditions legitimately fall within the classical dose-response concept, there is a developing suspicion, based on growing scientific evidence, that such doses introduce biases of considerable importance into the already difficult task of evaluating animal dose responses and the assessment of their relevance to human hazard identification and risk.[8] High doses which produce severe tissue damage (i.e., necrosis demyelination) and/or interfere in a significant manner with metabolic pathways or storage and excretion patterns in animal groups should be thought of as extremely toxic doses which can make interpretation difficult.

Responses produced by chemicals in man and experimental animals may differ according to the quantity of the substance received and the duration and frequency of exposure. In mammals, acute experimental exposure is usually thought of as a single exposure or multiple exposures occurring within twenty four hours or less. Such exposure, if the substance is rapidly absorbed, usually produces a mixture of responses. However, with this type of exposure, some toxic effects may be delayed (i.e., certain types of neurotoxicity, sensitization). Responses to acute exposures may be both qualitatively and quantitatively different from those produced by subchronic and chronic exposures and not all observed responses within a study, irrespective of exposure duration or frequency, will represent toxicity <u>per se</u>. They

will usually encompass a range of effects from physiologic through pharmacologic and toxicologic manifestations. Although it may be difficult at times to make a clear distinction between these responses, an attempt to do so must be made. When an evaluator is uncertain of the type or the biological significance of a response, he or she should not hesitate to obtain competent advice for resolving the uncertainty. It is essential that all relevant toxicity end points be identified for consideration when evaluating data for the presence or absence of nontoxic levels.

The following discussion presents the distinction, as made in this document, between three major response types - physiological, pharmacological, and toxic. Physiological responses vary within limits which are in accord with the normal functioning of a living organism. Examples of such response are the usual respiratory and pulse rate increases associated with increased physical activity; systemic changes associated with normal pregnancy, and those associated with homeostatic mechanisms. The variations in this type of response are usually referred to as "normal ranges" in clinical chemistry and other observational data. Generally these variable factors are not important toxicity end points in subchronic and chronic exposure studies unless their fluctuations are abnormally altered by a dose regimen. If such alterations occur at a specific dose or are part of a dose response relationship, they should be correlated with other toxicity end points which may be present.

Pharmacological responses are altered physiologic functions, are reversible, and are of relatively limited duration following removal of the stimulus. While some of these responses may be undesirable under certain circumstances, they are distinguished from toxic (adverse) responses by generally not causing injury. An example of this type of response is the increased activity of the hepatic cytochrome P-450 containing mono-oxygenase systems (enzyme induction) caused by exposure to many pesticides, industrial chemicals, and drugs.

Toxic responses may be reversible or irreversible but are distinguished from other types of responses by being injurious and therefore adverse and harmful to living organisms. The reversibility or irreversibility of a toxic response in animals and humans will depend on the ability of the injured organ or tissue to regenerate. For example, liver has a relatively great ability to regenerate and many types of injury to this organ are reversible. By contrast, differentiated cells of the central nervous system are not replaced and many types of injury to the CNS are irreversible.

An important concept, which has had several alterations in nomenclature over the last decade, is here designated as the "No Observed Effect Level" (NOEL). It is the dose level (quantity) of a substance administered to a group of experimental animals which demonstrates the absence of adverse effects observed or measured at higher dose levels. This NOEL should produce

no biologically significant differences between the group of chemically exposed animals and an unexposed control group of animals maintained under identical conditions.

Some implications of this definition need further discussion and elaboration. Its acceptability and usefulness depend entirely on the scientific rationale supporting the existence and demonstrability of a threshold for almost all responses produced by biologically active agents. As used here, the term "threshold" designates that level of a stimulus which comes just within the limits of perception, and below which level a recognizable response is not elicited. The earlier quotes of Paracelsus and the National Academy of Science are based on this fundamental concept. Its importance to the establishment of dose response relationships is discussed by Paynter.[8]

The National Research Council[10] has recently clarified the concept of risk assessment and distinguished two essential elements as follows:

> Regulatory actions are based on two distinct elements: risk assessment, the subject of this study, and risk management. Risk assessment is the use of the factual base to define the health effects of exposure of individuals or populations to hazardous materials and situations. Risk management is the process of weighing policy alternatives and selecting the most appropriate regulatory action, integrating the

results of risk assessment with engineering data and with social, economic, and political concerns to reach a decision. Risk assessments contain some or all of the following four steps:

° Hazard identification: The determination of whether a particular chemical is or is not causally linked to particular health effects.

° Dose-response assessment: The determination of the relation between the magnitude of exposure and the probability of occurrence of the health effects in question.

° Exposure assessment: The determination of the extent of human exposure before or after application of regulatory controls.

° Risk characterization: The description of the nature and often the magnitude of human risk, including attendant uncertainty.

In each step, a number of decision points (components) occur where risk to human health can only be inferred from the available evidence. Both scientific judgments and policy choices may be involved in selecting from among possible inferential bridges, and we have used the term risk assessment policy to differentiate those judg-

> ments and choices from the broader social and economic policy issues that are inherent in risk management decisions. At least some of the controversy surrounding regulatory actions has resulted from a blurring of the distinction between risk assessment policy and risk management policy.[10]

The concept of separating risk assessment and risk management functions, to the maximum extent feasible, allows evaluators to concentrate on analysis, evaluation, and interpretation of toxicological data according to sound scientific principles and without regard for what potential regulatory desisions or actions the results may portend.

B. Documentation and Data Acceptance

The quality, integrity, and completeness of reporting observational and experimental data are essential to the proper analysis and evaluation of submitted studies. In essence, the "good science" evaluations expected of EPA have their foundations in the submitted evidential documentation. Therefore, qualitative assessment of the acceptability of study reports has special significance for hazard identification and other aspects of risk assessment.

The following three important considerations address the acceptability of subchronic and chronic exposure studies and evidential documentation.

1. The adequacy of the experimental design and other experimental parameters such as: the appropriateness of the observational and experimental methods; frequency and duration of exposure; appropriateness of the species, strain, sex and age of the animals used; choice of doses, and the conditions under which the substance was tested.

There are no specific, internationally agreed upon scientific rules or fixed checklists which make the judgment regarding the acceptability of reports a standard routine procedure. However, there are suggested guidelines concerning the mechanics of good experimental design, reporting, and laboratory practice which are aids not only to the evaluation of report and data acceptability but also to the generation of scientifically valid data. These may be found in the OECD and EPA guidelines and the EPA and FDA Good Laboratory Practices Regulations.[1] However, the evaluator needs to be cautious when using the above guidelines as aids to making an acceptability judgment for any study. The cardinal question to be answered is how well does the study in toto facilitate the identification of potential adverse effects, or lack thereof, for the substance being evaluated, and not how precisely it fits a prescribed recipe for performance. The collective experience of HED evaluators can be very helpful in resolving difficult questions of acceptability and should be utilized whenever needed.

The evaluator should carefully read through the report including supporting data presentations, and make a tentative classification according to the Toxicology Branch Core Concept Manual. If there are obvious and significant deficiencies in the report which would lead the reviewing toxicologist to consider the study invalid, any further work would be a waste of resources. The submitter of the report should be notified, through the Product Manager, of the problem(s) as quickly and as accurately as possible and any further review suspended until these deficiencies are corrected.

Occasionally, the subsequent detailed analysis of the data will indicate deficiencies which were not obvious during the initial reading of the report. The deficiencies should be noted and the analysis completed as far as possible. The submitter of the document should be notified of the situation and provided with any scientific questions and other identified data needs.

2. The competency and completeness with which the study was conducted and reported.

Doubts on the part of the evaluator regarding the completeness and/or competency with which a study was performed or reported must be discussed with the evaluator's supervisor. If the doubts are judged to be reasonable, the study should be nominated for a laboratory and data base audit. Any further consideration of the study should be suspended until the audit is completed, reported, and evaluated.

3. The effects of modifying factors which result in major inequalities between control and treated animals.

This qualitative consideration has more to do with the evaluation and interpretation of data than with acceptability of documentation. It is placed here because determination of the various factors influencing toxicological data, as may be indicated in the submitted evidential documentation, needs to be made prior to the detailed data analysis.

There are many factors influencing the responses of experimental animals to chemical substances. Some of these are discussed by Doull[11] and his presentation of this subject should be reviewed. Some influences may be quite subtle as exemplified by studies performed by Thompson et al.[12] It had been noted that acute pulmonary edema occurred in rats being used in immune hypersensitivity studies and that the onset of this effect was sudden and seasonal. The onset was coincidental with hair-coat changes in laboratory rats as judged by shedding. Subsequent studies demonstrated that sulfur deficiency, which occurs seasonally in rats and which, according to the authors, primes the animal for pulmonary edema onset, also changes glucose and glycogen levels. The onset of acute pulmonary edema susceptibility was apparently due to seasonal alterations (hair-coat changes) in sulfur and carbohydrate metabolism as well as possible variations in insulin and other hormone levels. Circadian rhythms and seasonal physiological variations can subtly influence experimental

results. Also the presence of idiosyncratic responses or disease processes can complicate the evaluation and interpretation of any toxicity study. The factors influencing animal responses can be troublesome when their effects are confused with or misinterpreted as toxic. For further discussion of environmental effects on experimental parameters see Herrington and Nelbach.[13]

The three qualitative considerations discussed above are applicable to all experimental animal studies, no matter what their intended purpose, and essentially establish the acceptability not only of specific reports but also the acceptability of the eventual evaluation, interpretation, judgments, and risk assessments made by toxicologists.

Resolution of problems relating to qualitative or quantitative considerations is not entirely the responsibility of the individual evaluator. The submitter of the documentation may be requested to assist. For difficult problems, the assistance of consultants and/or the Science Advisory Panel may be utilized. Requests for the latter type of assistance must be made through the appropriate management level.

The acceptability of reports and other technical information submitted to EPA is primarily a scientific judgment and only secondarily a legal one. Therefore, EPA bears the burden of defending and documenting the acceptance or rejection, in part or

in whole, of the study report and data. The submitters of the information deserve to know the rationale for any rejection of data. The rationale should be succinctly stated in the evaluation document.

C. Major Considerations for Analysis and Evaluation

Control animals must receive as much attention during the analysis and evaluation process as do the treated ones. Any untreated (control) animal or group may exhibit some signs of abnormality or drift from the norm for that species or strain. Table 1, taken from Weil and Carpenter,[14] presents examples of abnormal values exhibited by control groups during long-term studies which could complicate analyses of data. Because of the real possibility that statistically significant differences between chemically treated and untreated control groups are the result of abnormal values among the controls, the authors concluded that to be indicative of a true deleterious (adverse) effect, the differences should be dose-related and should delineate a trend away from the norm for that stock of animals.

Historical control data is useful when evaluating the acceptability of the "normal" values and observational data obtained from control groups.[8,15,16,17] Any departure from the norm by the control group(s) must be discussed in the evaluation document and taken into consideration, especially during any statistical analysis.

Weil and McCollister[18] analyzed toxicity end points, other than oncogenicity, from short- and long-term tests and concluded that only a relatively small number of end points are effective in delineating the lowest dosage producing an effect in such tests. Body weight, liver weight, kidney weight, and liver pathology delineated this dosage level in 92% of test chemicals in short-term (subchronic) studies and 100% in long-term (chronic) studies. To reach 100% efficiency in short-term studies, renal and testicular histopathology had to be included. There is no implication that these criteria delineate all of the stress markers or toxicity end points likely to appear at higher dose levels. However, it is implied that toxicity effects in these data areas are likely to appear earlier in a study and at a lower dose than many other markers. Heywood[19] surveyed the toxicological profiles of fifty compounds in rodent and non-rodent species and confirmed the observations of Weil and McCollister. For this reason these criteria of stress should receive careful attention in the analysis and evaluation process.

1. Mortality/Survival

Death is a highly definitive, biological end point for analysis regardless of the animal group or groups in which it is observed. Reasonable efforts should be made to determine the cause of individual deaths or to discover a defensible presumption of the cause. The evaluation of pathological lesions or morphological changes in unscheduled, belatedly observed deaths are very frequently

complicated or hampered by postmortem autolysis. The separation of deaths caused by factors unrelated to pesticidal agent exposure (e.g., acute or chronic infections, age or disease dependent degenerative processes, anatomical abnormalities, negligent handling or accident) from toxicity induced deaths is important. All data relating to the moribund or dead animals during their study life, as well as the results of postmortem examinations, should be scrutinized in an attempt to make this distinction.

Mortality analysis requires more than a statistical treatment of incidence at termination of a study (e.g., Example A, Table 1). Survival/mortality data can be influenced by many factors other than toxicity of the test substance. Changes in protocols during the course of a study can complicate the analysis. Alterations in dosage levels can produce a confusing mortality pattern. This is also true of kills and especially unscheduled kills during a study. The perturbation caused by both types of changes during a study can be considerable and the resolution of difficulties may not be a simple routine.[8]

Any unusual mortality pattern should be explained by the data submitter on biological or toxicological grounds. If mortality is high *in toto* for any short- or long-term study, or for a particular group within a study and a credible explanation is not available, the study should be nominated for a laboratory and data base audit.

An analysis and evaluation of mortality patterns within each group is important. Such patterns may indicate mortality is clustered early or late in the course of the study; is intermittent and scattered throughout the duration of the study; or has a higher incidence in one sex than in the other. The analysis of the cause of individual deaths will aid in determining the toxicological significance of these various patterns. Early deaths within treated groups (i.e., those occurring within the first eight weeks of a subchronic study or within the first ten months of a chronic study), can provide very valuable information because they may represent the more susceptible animals among the exposed population. However, Fitzhugh et al.,[20] found that when the quantity of test substance in the diet is kept constant, young rats ingest relatively more of the test substance than do older rats. This growth dilution phenomenon is illustrated for male rats in Figure 1 and for females in Figure 2. Compound consumption, in mg/kg body weight per day, for each of the first 13 weeks and selected intervals thereafter is also presented for males (Figure 1A) and females (Figure 2A). In these illustrations it can be seen that for the first 13 weeks, a rapid weight gain period for both sexes, the mg/kg of body weight per day consumption of the compound is relatively high and tapers off to a relatively stable value at approximately 40 weeks. Early deaths may therefore be the result of the higher exposure, on mg/kg/day basis, of young animals compared to older animals. Deaths which are clustered at a specific time period may reflect a spontaneous epidemic disease situation of limited duration. However, high mortality

associated with infectious agents in treated groups, in the absence of such evidence in the concurrent control group, may portend an immuno-suppressive action on the part of the chemical being tested.

2. Clinical Observations

Generally, adverse clinical signs noted during the exposure period should correlate with toxicity end points or disease processes. These can frequently be used as supportive evidence for dose-response-relationships and can play an important role in determining the NOEL. However, not all adverse clinical signs will correlate with pathological or morphological changes in organs or tissues. Some will be caused by biochemical lesions or shifts in mechanisms which require special methods for their detection (i.e., incoordination, muscle twitching, tremor, or diarrhea may indicate acetylcholinesterase inhibition without any morphological changes being evident in nerve tissue).

Table 2 presents some of the clinical signs which may be observed during the physical examination of individual animals. Very few of these observations are made with the aid of instruments. It is, therefore, essential that all deviations from the "normal" observed in the control and treatment groups be adequately and accurately described and recorded during the study and presented, in like manner, in the study report.

Many of these qualitative signs can be counted, scored for intensity, and tabulated as incidences. However, statistical analysis is not of any real value in this area. The evaluator

must, therefore, rely more on the number of individuals per group exhibiting signs of a particular type, as well as the intensity of the reponses, to gain an impression of a dose-response-relationship.

Clinical observations such as those that relate to palpable tumors or which might be associated with neoplastic developments such as hematuria, abdominal distention, or impaired respiration may be useful in defining the time a tumor was first suspected as being present. Such signs might be an aide in evaluation of decreased tumor latency in long-term rodent studies. They may also aid in determining cause of death. A statement of the correlations, or the lack thereof, between clinical signs and specific toxicity end points should be made in the evaluation document.

3. Body Weight and Food Consumption

Body weight changes (gains or losses) for individual animals and groups of animals when compared to concurrent control changes during the course of a study are a criterion of some importance.[18,19,22] Such changes are usually related to food intake and analysis of one without an analysis of the other is of little value. Weight decrement may not always be related to toxicity per se.[23] Occasionally the incorporation of the test substance into the diet will cause the diet to be unacceptable (unpleasant or not palatable) to many individuals in all treatment groups or to the majority of individuals in the higher dietary level groups.

This effect is usually evident during the first two or three weeks of the study. Sometimes the majority of animals in the affected groups(s) are able to accomodate and a gradual increase in group weight gain will occur.[24] In subchronic studies, the lag in group weight gain may persist, even though the individual animal gains per gram of food consumed (food efficiency) are favorable after the accommodation, and produce a statistically significant difference between the affected group and the concurrent controls which is not related to toxicity of the test substance.[25] This phenomenon is infrequently encountered in chronic studies, since the problem can usually be overcome by an appropriate method (e.g., intubation). Sometimes the addition of the test substance will interact with one or more essential nutritional elements in the diet thereby producing weight gain decrements or alterations of toxic responses.[26,27,28] This phenomenon may be encountered in subchronic studies and when identified is usually overcome by acceptable means before a chronic study is initiated. Infrequently seen is the control effect illustrated by Example B in Table 1. This data represents a situation in dogs where the control value is very low causing the other value to appear unusually high, but it can be encountered in rodents, where at one point in time the controls exhibit an unusual weight difference when compared to the treated groups.

Diet composition, food consumption, and body weight gains *per se* can also have an important influence on many aspects of animal responses including shifts in metabolic, hormonal, and homeostatic

mechanisms[29] as well as disease processes[8,30,31,32] and maturation[33] and should be considered when unusual effects are observed in the absence of any indication of injury to organs and other vital systems.

The resolution of difficulties in evaluation of body weight changes and attendant effects may be aided by the graphing of group body weight and food consumption and compound consumption (on a mg/kg body weight basis). This allows a quick identification of any unusual or sudden changes in gain or loss by any group. In any case the evaluator should do some independent analysis of body weight differences to determine whether an agreement or disagreement with the submitters' conclusion or opinion can be reached in an independent and defensible manner.

4. Hematologic, Clinical Chemistry, and Urinary Measurements

The Pesticide Assessment Guidelines, Subdivision F, suggest that certain measurements of hematologic, clinical chemistry, and urinary parameters be routinely made in rodent and non-rodent subchronic[3] and chronic[4] toxicity studies.

There is little doubt about the value to clinicians of such data when treating or otherwise managing human and veterinary patients and such data may also be of value to pesticide toxicologists when subchronic studies are being used to establish dose regimens for longer term studies. Because of the automation of both the routine clinical analysis and the statistical treatment

of this type of data, evaluators will be forced to contend with much "noise" in this area, and will frequently be presented with scattered, statistically significant effects in the absence of any evidence of clinically significant relationships to specific toxicity end points. For example, Pearl et al.,[34] restrained rats for six hours and followed SGOT and SGPT changes. These transaminases were very much elevated and the SGOT did not return to basal level within a period of six days, indicating an apparent susceptability of these enzymes, particularly SGOT, to stress factors.

Table 1, Examples C and D, presents examples of statistically significant differences of lymphocyte counts and serum urea nitrogen determinations which are not biologically significant because of the control effect mentioned previously. These data also illustrated the frequently observed random occurrence and non-dose-relationships of this type of data. When using historical control data as an aid to evaluation, it must be kept firmly in mind that "normal values" in hematologic and clinical chemical measurements depend heavily on the specific methods used to generate the data. Therefore, only values produced by the identical methods from the same laboratory are valid in such comparisons. Literature values for normal ranges which do not specify the method by which they were obtained must be used with caution.

Blood cytological and chemical data, with urinalysis, can be valuable information in toxicity testing. Heywood,[19] in

surveying the correlation of sensitive criteria of target organ toxicity across species, found that reduction of values relating to red blood cells was a common effect recorded in all species in his survey when the hemopoietic system was affected. Interim elevations in serum enzyme levels of aspartate transminase (SGOT or AST), alanine transaminase (SGPT or ALT) and alkaline phosphatase may be predictive of potential or actual hepatic lesions, but should be confirmed by histopathological changes. Measurement of specific isoenzymes of alkaline phosphatase may help distinguish the site of a lesion, (i.e., bone, liver, placenta or intestine). AST elevations may also suggest cardiac degeneration. Stress and injury to the kidney may be reflected in increases in blood urea nitrogen and creatinine levels which are generally correlated with urinalysis data. Evaluation of lactic dehydrogenase may indicate liver or cardiac injury and other myopathies. Another indicator of cardiac or skeletal muscle lesions is an increase of serum level of creatine phosphokinase. It is important to understand that many of these types of serum enzyme tests and urinalysis fail to detect minor injury or may reflect only transient or reversible changes. Therefore, evaluation and interpretation of the test results must be performed carefully and correlated with more specific, sensitive, and reliable histopathologic findings. Plaa[35] discusses the conversion of liver function data into quantal responses as well as the quantitative problems involved in low-frequency adverse reactions and the difficulty these present in the detection of liver injury

in laboratory animals.

Sensitivity and specificity of the enzyme changes as diagnostic of organ pathology are greatly influenced by the species selected for testing.[36] For example, in mammalian species, aspartate transminase is not specific to any tissue and thereby elevated plasma AST activity may suggest damage to any one or many tissues. In contrast, alanine transaminase is relatively specific to the liver in the cat, dog, ferret, mouse, and rat, whereas in primates, ALT is present in heart, skeletal muscle, and liver. Plasma alkaline phosphatase measurement has been less useful in detecting liver cell necrosis in the dog, sheep, cow, and rat but may be indicative of other types of liver damage, particularly those of a cholestatic nature in a number of species. It is evident that species differences are of great importance when specific clincial chemistries are being selected for inclusion in toxicity studies.

When analysis and evaluation of clinical data indicate a dose response relationship or a biologically important drift from concurrent control values, the effects observed must be correlated with other manifestation of toxicity. The evaluator should also state that a correlation could not be made when that is the situation.

Standard References (e.g., Reference 37) should be consulted for evaluation of potential correlations between clinical chemistry, hematologic, urinary data, and adverse effects.

5. Organ Weights and Body Weight Ratios

Current EPA guideline protocols recommend that at least liver, kidney and testes be weighed during necropsy of animals in sub-chronic exposure studies[3] and that, in addition to these, brain weights be determined in chronic toxicity studies.[4] The most efficient criteria, according to Weil and McCollister,[18] and Heywood,[19] for evaluation of the lowest dosage producing an effect in such studies are changes in liver, kidney, and body weights.

Organ weight is usually reported both with and without a consideration of body weight. The former is referred to as absolute organ weight and the latter as relative organ weight. Relative organ weight comparison is especially useful when body weight is effected in a compound-related manner. Experimentally controllable and uncontrollable factors (i.e., circadian rhythms, food intake, nature of the diet, age of animals, organ workload, stress, and method of killing) have an influence on organ and body weights and the variability of such data. A review of this subject, by Weil,[38] should be read by all evaluators. The most important influencing factor appears to be the method of killing and the timing of necropsy. The killing method used not only affects the appearance of the tissue, important in describing gross necropsy observations, but also, in conjunction with the timing of necropsies, may cause postmortem shifts in organ weights.[39,40] A uniform exsanguination technique has been described and evaluated by Kaneva, et al.,[41] which significantly ($P<0.05$) reduced the absolute and relative liver and kidney weights with

respect to these weights from animals that were not exsanquinated. The standard deviations of the mean absolute and relative liver weights were also significantly ($P<0.05$) reduced. Exsanguination, in this study, did not appear to affect the absolute or relative weights nor the standard deviations for heart, brain, and spleen. Additionally, the use of fasted animal body weights can reduce the variability of organ/body weight ratios. Adkins, et al.,[42] discuss the standardization of the technique for determination of testes weights to reduce variability.

The interpretation of organ weight changes must not be made solely on the determination of a statistically significant difference between the concurrent control value and a treatment group value. A proper evaluation will also include consideration of any correlation between organ weights, histopathologic and metabolic/pharmacodynamic data. Such correlations if they exist must be discussed in the evaluation documentation.

6. Postmortem Observation

The pathologist has a unique position in toxicological and oncological evaluations. Such individuals perform a special role in providing information on the differences in tissue and organ morphology that will establish the presence or absence of dose effect relationships for some lesions. This data is critical to establishment of toxic and other effects produced by a substance. Zbinden[43] discusses the role of the pathologist in some detail. He also discusses the use of semi-quantitative

methods as well as more accurate morphometric methods for rating the severity of lesions, but cautions that even with their use, we cannot be entirely satisfied with diagnostic labels for lesions because of the lack of generally and internationally accepted nomenclature in toxicological pathology. The problems created by differing nomenclature are also discussed by Haseman, et al.[17] To prevent this type of problem, an experienced pathologist will describe each significant lesion type, at least once, in such detail that any competent pathologist can perceive a good mental picture of the lesion and form his own judgment as to its relevance to the histopathology induced by the chemical being tested.

More detailed discussions of problems relating to complete reliance in diagnostic terms and other aspects of evaluating oncogenic potential are presented by Paynter.[8] Age associated, especially geriatric, influences can have an extremely important effect on histopathologic as well as clinical chemistry, metabolic and pharmacokinetic data bases;[44] and therefore important overt, and frequently subtle, influences on observed physiologic, pharmacologic, and toxicologic response during the latter part of any long-term study. As indicated earlier, spontaneous degenerative lesions, especially when misinterpreted as induced toxic effects, can cause major difficulties in hazard evaluation and risk assessment. It is essential in all cases where spontaneous and/or age associated lesions are present, to differentiate between such lesions and treatment induced lesions. References such as Grice and Burek[44], Benirschke and Jones[45] are very helpful in this respect but are really not a substitute for

advice from a competent and experienced pathologist. For detailed descriptions of potential histopathological changes induced by toxic substances, spontaneous or degenerative and other diseases, and their incidences in experimental animals, see Reference 45.

D. Consideration of Auxiliary Evidence

The usefulness of mammalian metabolism data and the enhancement of our knowledge of response mechanisms by studies of absorption, distribution and elimination patterns of a test substance is briefly discussed by Paynter.[8] The following references cited in that document are of importance to the evaluation and interpretation of subchronic and chronic exposure study data: Wolf (1980), Anderson (1981), Smith and Hottendorf (1980), Yacobi et al. (1982), Park (1982), and Mitchel et al. (1982).

In addition, references in this document discuss dose-dependent effects in the absorption process and biotransformation interactions;[46] the potential difficulties presented by impurities and the overloading of detoxification mechanisms;[47] and various other important aspects of experimental considerations.[48]

E. Completion of Analysis

At this point an evaluator should have formulated judgments and supporting rationale concerning: a) the acceptability of the data base; b) the existence of biologically important toxic and/or oncogenic effects, c) the relevancy of any modifying factors; and d) the likelihood that any of the observed effects were induced by the administered substance.

The evaluator should summarize, briefly and cogently, the critical biological and auxiliary data together with any modifying factors for all studies under review. Any rationale pertinent to an evaluation of the toxic and oncogenic potential of the substance should also be included in the summary. NOEL's or the absence thereof, should be clearly stated for each of the critical biological and toxicological responses noted.

II. Evaluation of Weight-of-Evidence

The essential purpose of subchronic and chronic exposure studies is the detection of valid biological evidence of the toxic and/or oncogenic potential of the substance being investigated. In this document, the evaluation of the strength or weight of evidence produced by toxicity studies is that process which considers the cumulative observational and experimental data pertinent to arriving at a level of concern about a substance's potential adverse effects. It is composed of a series of judgments concerning the adequacy, validity, and appropriateness of the observational and experimental methods used to produce the data base, and those judgments which bring into causal, complementary, parallel, or reciprocal relationships, all the data considered. Because our knowledge concerning toxic mechanisms is still developing, because good epidemiological evidence is seldom available, and because animal studies are not always conclusive; all of the information available at a given time may provide only "persuasive evidence" (i.e., not clearly robust; feeble), suggestive of a defensible presumption one way or another about

the potential health effects of a substance under given conditions of exposure. It is therefore necessary to succinctly articulate the rationale for judgments and conclusions contained in risk assessments and the uncertainties pertaining thereto. This becomes important when new data or new scientific knowledge requires reevaluation of the data base or a change in a previous risk assessment or regulatory action.

For the present there is no acceptable substitute for informed judgment based on sound scientific principles in analyzing, evaluating, interpreting, and weighing biological and toxicological data derived from currently available animal toxicity study protocols. The present universally accepted practice of estimating a NOEL in subchronic and chronic animal studies is based on the following procedure: (1) Identification of adverse effects induced by a known quantity of a chemically and physically characterized substance. Generally, a defensible presumption that the observed adverse effects are induced by a known exposure to the substance is based primarily on the detection of a trend away from the normal for the species and strain of animals used (concurrent control and/or historical control data) and a demonstration of a dose-response relationship for an observed effect or spectrum of effects; (2) Identification of an approximate threshold level where the adverse effects observed at higher doses are just perceptable (the lowest adverse effect level); and, (3) Identification of a dose level which does not elicit the adverse effects

observed at the threshold or higher levels (i.e., absence of adverse effects). This includes the judgment that any other effects observed at this level portend no biologically significant consequences for the health and well being of the exposed population.

It is also a universally accepted practice to apply uncertainity factors to the NOEL derived from subchronic and chronic animal studies when estimating a guide post, i.e., ADI as an aid in evaluating the acceptability of actual or potential human exposure limits. For further discussion of this subject see Weil,[49] Paynter and Schmitt,[50] and Dourson and Stara.[51] The development of mathematical models,[52,53] may modify this process in the future.

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Table 1
Abnormal Values In Control Groups*

Example A
Mortality of Rats[a]

Chemical in diet (g/kg)	Mortality	
	Ratio	Percentage
0.50	9/15[b]	60
0.10	8/14[b]	57
0.02	8/17[c]	47
0.00	15/15	100

[a]Mortality of rats alive at 1.5 year of doses during last half-year of inclusion of UCON lubricant 50-HB-5100 in the diet of rats for 2 years.
[b]0.05>P>0.01 [c]0.01>P>0.001

Example B
Body Weight Gain of Dogs[a]

Chemical in diet (ppm)	Mean body weight chan (kg)
6400	1.08[b]
1600	0.70
400	0.70
0	0.03

[a] Weight change during inclusion of TERGIT anionic 08 in the diet of dogs for 1 yea
[b]0.05>P>0.01.

Example C
Percentage of Lymphocytes in Dogs[a]

Number of doses	Chemical in diet	
	100 ppm	0 ppm
0	30.8	32.6
59	32.5	40.5
128	35.5	29.5
155	32.9	30.5
185	33.5[b]	18.2
249	34.1	33.2
	Percentage of group mean[c]	
185	101	55

[a]Data from white cell differential blood count during the inclusion of CRAG SEVIN insecticide in the diet of dogs for 2 years (200 cells counted).
[b]0.05>P>0.01.
[c]Group mean does not include value at significant period.

Example D
Serum Urea Nitrogen in Dogs[a]

Number of doses	Chemical in diet (g/kg)	
	0.009	0.000
0	23.9	26.1
67	24.1	21.2
138	22.8[b]	17.0
195	25.9	21.5
209	19.7	17.6
243	20.0	18.3
255	22.4[b]	16.7
261	24.2	20.0
	Percentage of group mean[c]	
138	99	82
255	98	80

[a]Weight change during inclusion of TERGITOL anionic 08 in the diet of dogs for 1 year
[b]0.05.P>0.01.
[c]Group means do not include values at significant periods.

Example E
Tumor Incidence of Rats[a]

Chemical	Female rats with tumors	
	Ratio	Percentage
0.50	4/18[b]	22
0.10	12/20	60
0.02	8/18	44
0.00	16/20	80

[a]Tumors in female rats during second year of inclusion of UCON lubricant 25-H-2005 in the diet of rats.
[b]0.01<P>0.001.

Table 2

Physical examination in toxicity tests in rodents.

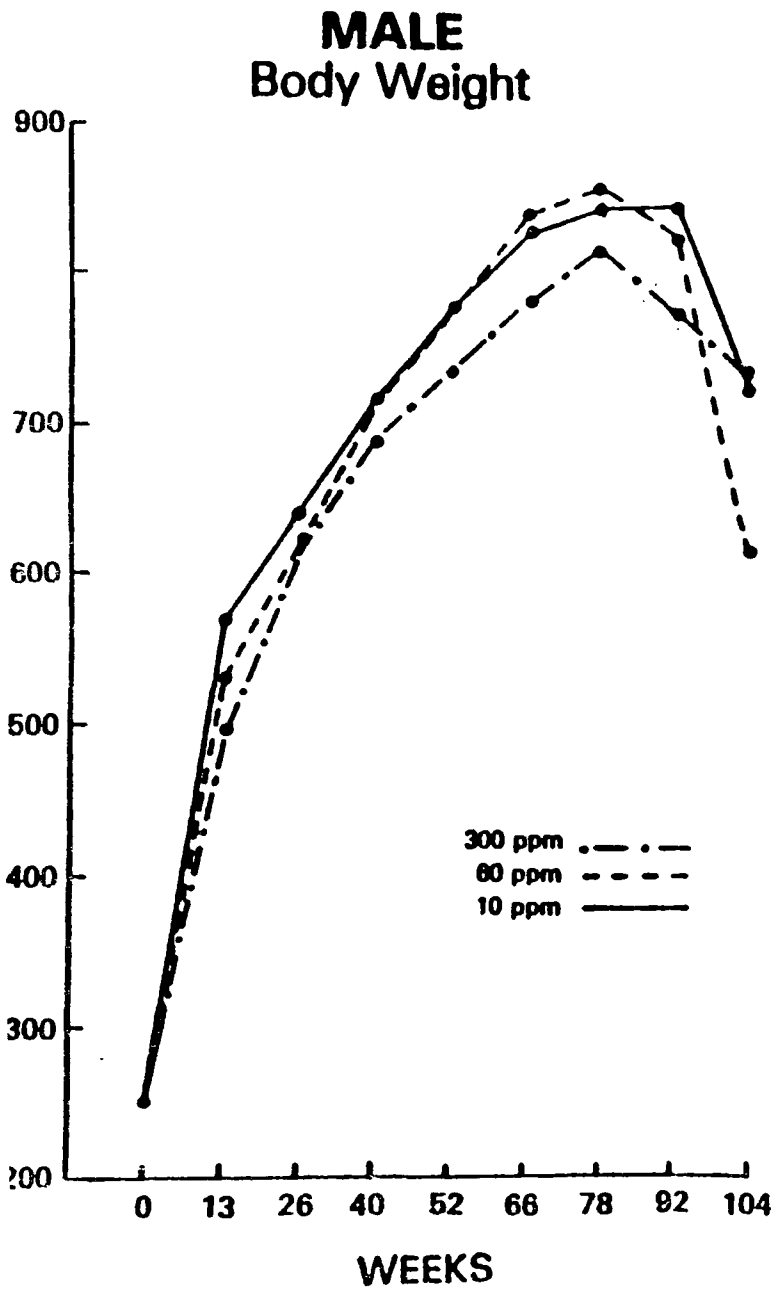
Organ system	Observation and examination	Common signs of toxicity
CNS and somatomotor	Behaviour	Change in attitude to observer, unusual vocalization, restlessness, sedation
	Movements	Twitch, tremor, atoxia, catatonia, paralysis, convulsion, forced movements
	Reactivity to various stimuli	Irritability, passivity, anaesthesia, hyperawsthesia
	Cerebrial and spinal reflexes	Sluggishness, absence
	Muscle tone	Rigidity, flaccidity
Autonomic nervous system	Pupil size	Myosis, mydriasis
	Secretion	Salivation, lacrimation
Respiratory	Nostrils	Discharge
	Character and rate of breathing	Bradypnoea, dyspnoea, Cheyne-Stokes breathing, Kussmaul breathing
Cardiovasular	Palpation of cardiac region	Thrill, bradycardia, arrhythmia, stronger or weaker beat
Gastrointestinal	Events	Diarrhoea, constipation
	Abdominal shape	Flatulence, contraction
	Faeces consistency and colour	Unformed, black or clay coloured
Genitourinary	Vulva, mammary glands	Swelling
	Penis	Prolapse
	Perineal region	Soiled
Skin and fur	Colour, turgor, integrity	Reddening, flaccid skinfold, eruptions, piloerection
Mucous membranes	Conjunctiva, mouth	Discharge, congestion, haemorrhage cyanosis, jaundice
Eye	Eyelids	Ptosis
	Eyeball	Exophthalmus, nystagmus
	Transparency	Opacities
Other	Rectal or paw skin temperature	Subnormal, increased
	Injection site	Swelling
	General condition	Abnormal posture, emaciation

* From (21) Table 3.1, p 53

Figure 1.

Male Rat Body Weight in Grams and Compound Consumption in mg/kg of Body Weight/day

The values for the selected weeks, in the compound consumption graph, represent the percent of the first week compound consumption.


MALE
Body Weight
900
700
600
500
400
300
200
0
13
26
40
52
66
78
92
104
WEEKS
300 ppm
60 ppm
10 ppm

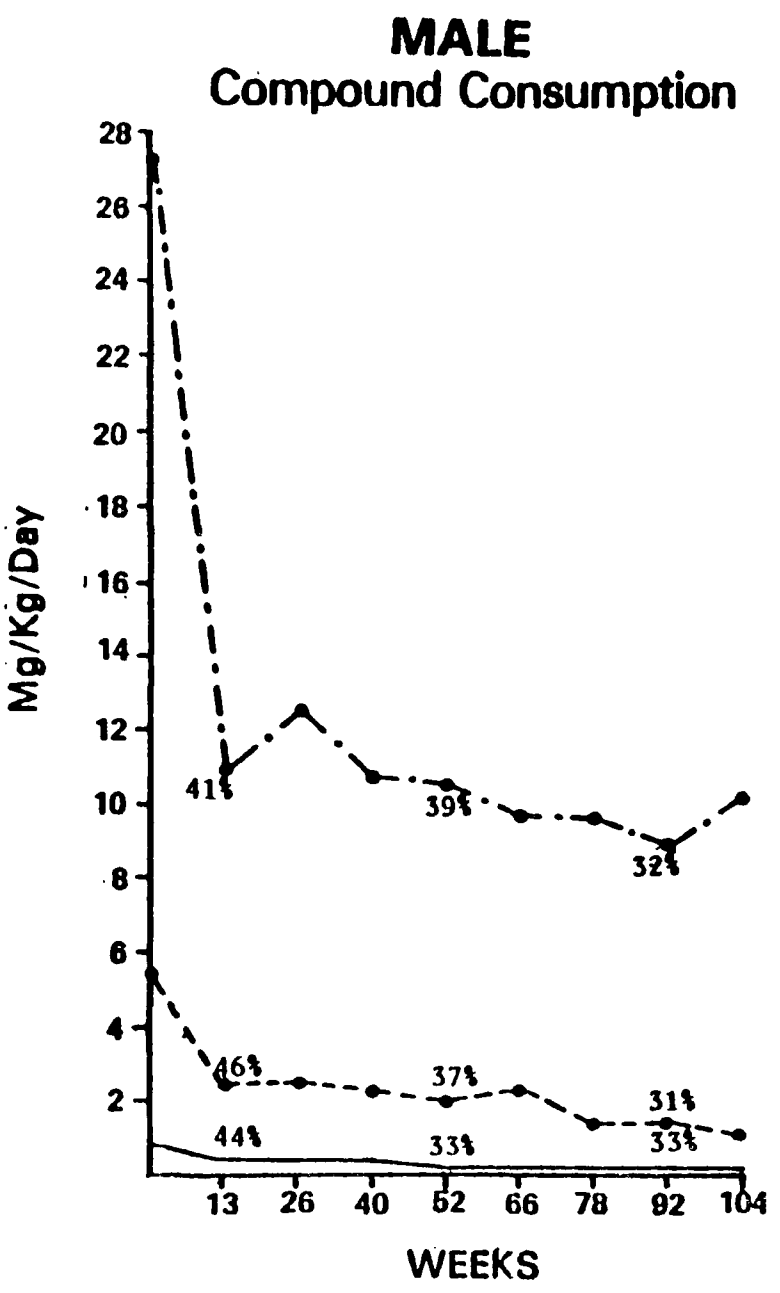
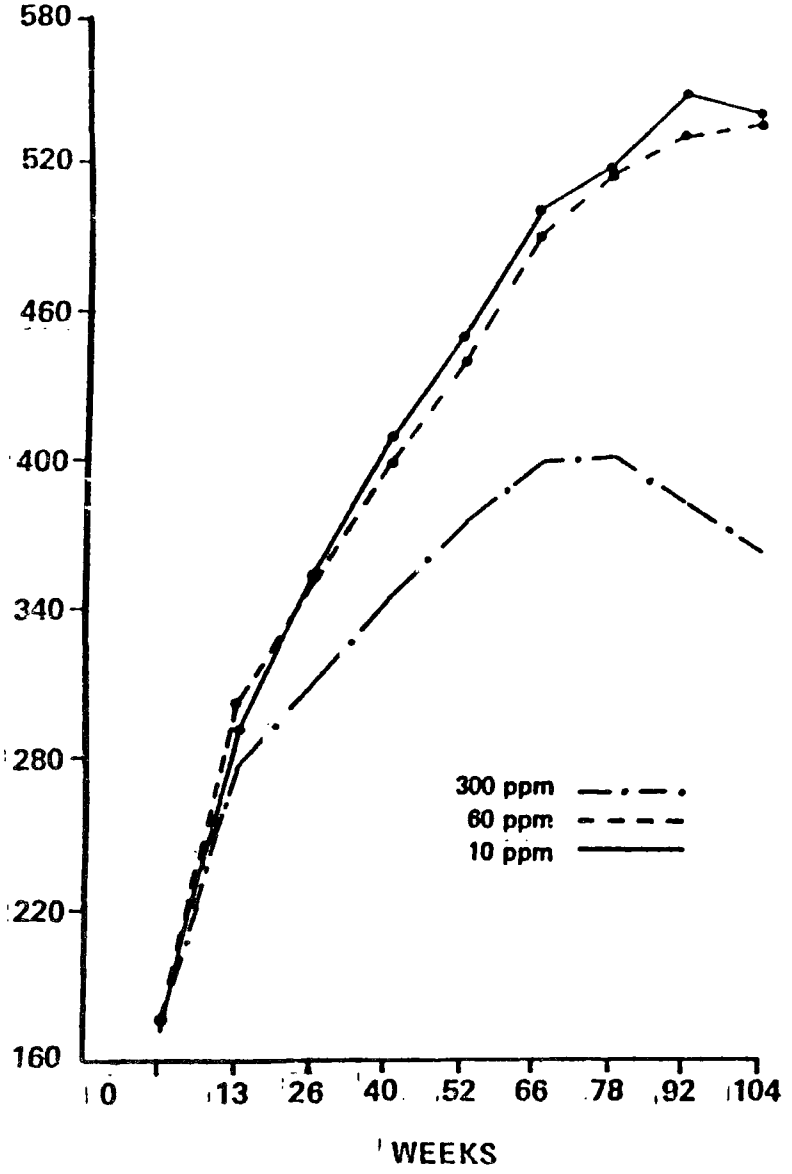

MALE
Compound Consumption
Mg/Kg/Day
28
26
24
22
20
18
16
14
12
10
8
6
4
2
41%
39%
32%
46%
37%
31%
44%
33%
33%
13
26
40
52
66
78
92
104
WEEKS

Figure 2.

Female Rat Body Weight in Grams and Compound Consumption in mg/kg of body weight/day

The values for the selected weeks, in the compound consumption graph, represent the percent of the first week compound consumption.

FEMALE
Body Weight



FEMALE
Compound Consumption

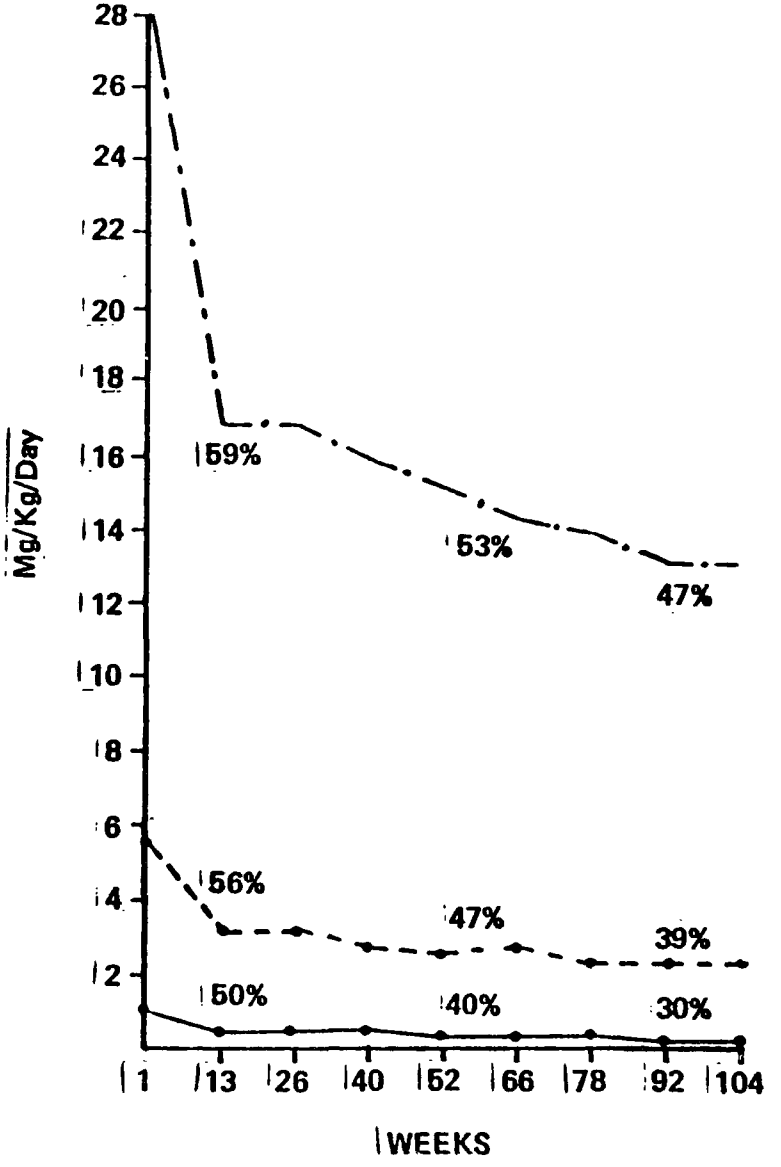


Figure 2

Figure 1A

Male Rat Compound Consumption in mg/kg/of body weight/day

The values for the selected weeks represent the percent of the first week compound consumption.

Figure 2A

Female Rat Compound Consumption in mg/kg of body weight/day

The values for selected weeks represent the percent of the first weeks compound consumption.

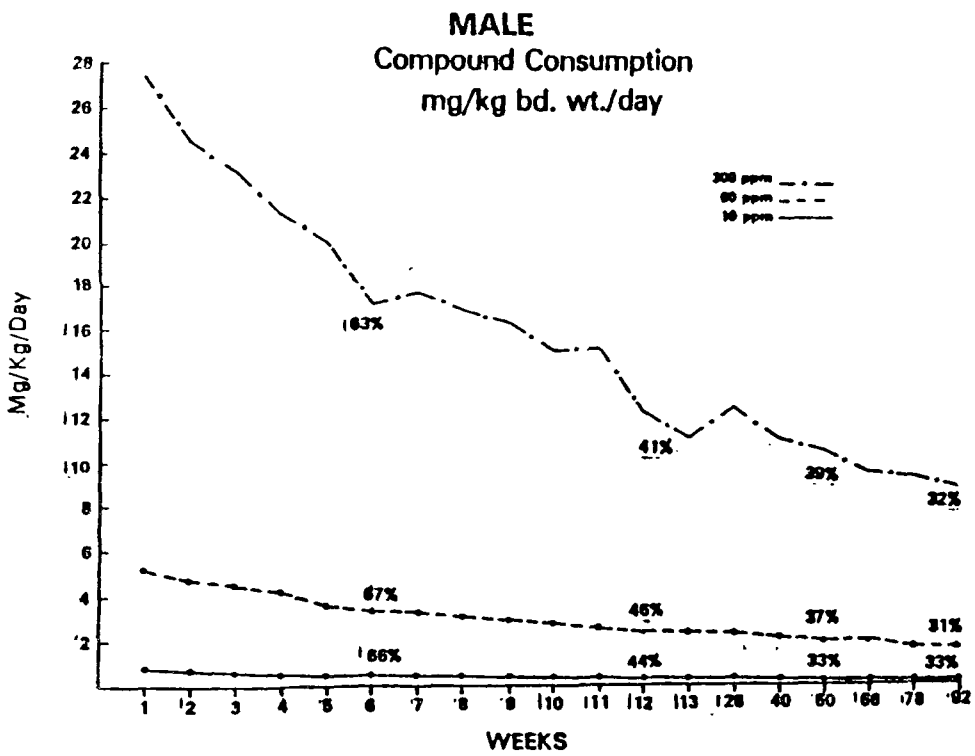


Figure 1A

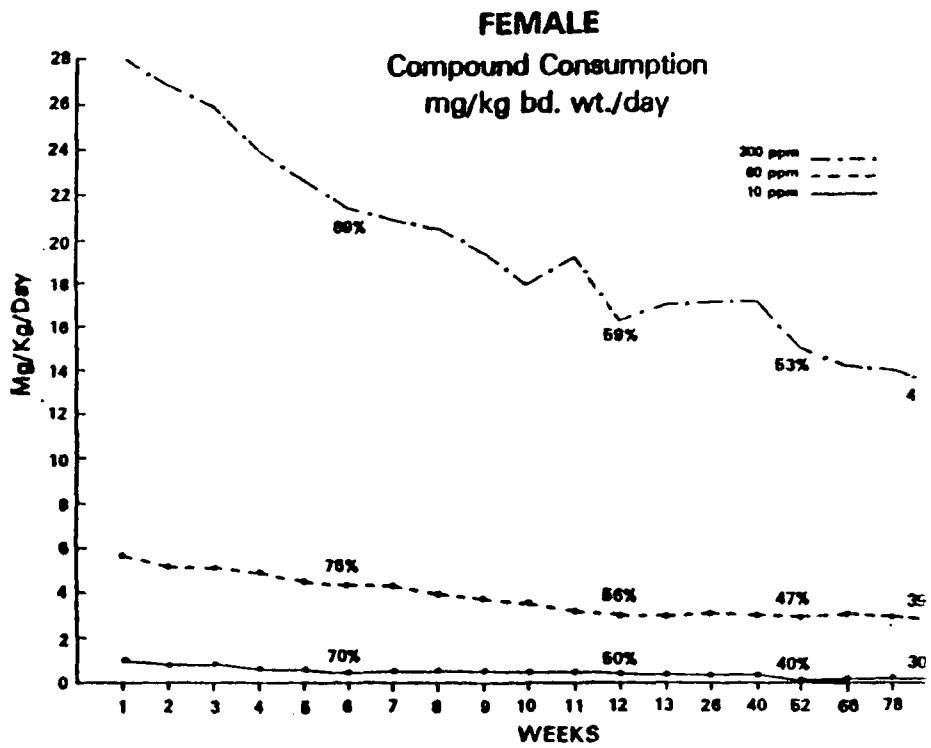


Figure 2 A